

Re-description of *Xyliphius barbatus* (Siluriformes, Aspredinidae), with comments on osteology and distribution

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Abstract

The banjo catfish, *Xyliphius barbatus*, belongs to the Aspredinidae family and typically inhabits the main channels of medium to large rivers in the La Plata River basin. The mimetic coloration with the substrate and the benthic lifestyle likely contribute to the challenge of sampling this species, resulting in its underrepresentation in museums and ichthyological collections. In fact, the original description of *X. barbatus* was based solely on two specimens. Consequently, little is known about its osteology, distribution, and phylogenetic relations. In this work, these information gaps are filled and the distributional range for *X. barbatus* is extended to northwestern Argentina.

Key Words

Banjo catfish, fossorial fishes, La Plata River basin, morphology, osteology

Introduction

The family Aspredinidae is composed of 13 genera and 49 valid species (Fricke et al. 2023a), among which the genus *Xyliphius* Eigenmann, 1912, include seven species (Fricke et al. 2023b). According to Friel (1994) the genus is distinguishable from all other aspredinids by the following combination of characters: eyes highly reduced, premaxillary toothless and displaced lateral to mesethmoid, a row of fleshy papillae on lower lip, *unculi* and *unculiferous* tubercles flattened, lamina of pterotic

rounded, and lateral end of posterohyal expanded. Members of this genus are distributed in different basins in South America: *X. sofiae* Sabaj Pérez, Carvalho & Reis, 2017 in the Amazon River basin in Peru, *X. melanopterus* Orcés, 1962 and *X. lepturus* Orcés, 1962 in the upper Amazon and Orinoco River basins, *X. kryptos* Taphorn & Lilyestrom, 1983 in the Maracaibo basin, *X. anachoretas* Figueiredo & Britto, 2010 in the Tocantins River basin, *X. magdalenae* Eigenmann, 1912 in the Magdalena River basin, and *X. barbatus* Alonso de Arámburu & Arámburu, 1962 from La Plata River basin.

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Except for *Xylophius lepturus*, all the species of the genus were described based on one to three specimens (*X. lombarderoi*, *X. melanopterus*, *X. magdalenae* and *X. sofiae* based on a single specimen; *X. barbatus* and *X. anachoretas* on two specimens; and *X. kryptos* on three specimens). These fishes are rare in inventories of fish fauna and consequently in museum collections. This sparse representation is due to several factors, including their mimetic coloration with the substrate, their benthic lifestyle, the infaunal habits of many species, and, most importantly, their specific habitat preferences. Indeed, these species live in the main channels of medium to large rivers, where sampling is difficult (Carvalho et al. 2017). *Xylophius barbatus*, originally described from the Paraná River in Rosario (Alonso de Arámburu and Arámburu 1962), has a few records along the La Plata River basin: Paraná River in Misiones, Santa Fe (García 1992; Calviño and Castello 2008) and Chaco provinces (type locality of *Xylophius lombarderoi* Risso & Risso, 1964, a synonym of *Xylophius barbatus*) (Risso and de Risso, 1964); in the Uruguay River in Uruguay (Loureiro et al. 2013) and, more recently, in the Paraguay River basin, Pantanal, Brazil (Gimênes Junior and Rech 2022).

In Argentina, other aspredinids have been recorded: *Amaralia oviraptor* Friel & Carvalho, 2016, *Bunocephalus doriae* Boulenger, 1902, *Pseudobunocephalus iheringii* (Boulenger, 1891), *Pseudobunocephalus rugosus* (Eigenmann & Kennedy, 1903), and *Pterobunocephalus depressus* (Haseman, 1911). Although the upper section of the Bermejo River basin has many endemisms (e.g., Mirande et al. 2004a, b, 2006; Casciotta and Almirón 2004; Terán et al. 2016a, 2019; Alonso et al. 2018; Aguilera et al. 2019), it shares elements with the Paraguay-Paraná River basin (Alonso et al. 2016; Terán et al. 2016b, 2016c; Vanegas-Ríos et al. 2019), and the Amazon River basins (Littmann et al. 2015; Aguilera et al. 2022). The only record of aspredinids in this area is *Bunocephalus doriae* Aguilera et al. (2016).

New inventories made in the Bermejo River basin have revealed the presence of *Xylophius barbatus*, a species not previously recorded for the area. This record is the largest known batch regarding the species. The aim of this contribution, is to provide an accurate re-description of *X. barbatus*.

Materials and methods

Specimens were collected by electrofishing and hand nets, euthanized by immersion in tricaine methanesulfonate (MS222), fixed in 10% formalin solution, and transferred to individual batches in a 70% alcohol solution. The material was deposited in ichthyological collections. In some fresh individuals, a small tissue sample was taken and immediately preserved in absolute ethanol for genetic analysis. The tissue aliquots were deposited in the

tissue collection of CIT-FML. The study complied with the animal welfare laws, guidelines, and policies of the Comité Nacional de Ética en la Ciencia y Tecnología, Argentina. Collection permits were granted by the Ministerio de Ambiente of Jujuy. (permits' numbers: 1103-306-M/2016, Res. N° 137/2016-MA).

Point to point measurements were taken with a digital caliper to the nearest 0.1 mm and expressed as percentage of the standard length (SL), except for subunits of head, expressed as percent of the head length (HL) (see Table 1). Measurements follow Friel (1995) and Cardoso (2010), with the modifications proposed by Carvalho et al. (2017). Nomenclature of anatomical structures follows that used by the three mentioned authors and Dahdul et al. (2010), included in the Teleost Anatomy Ontology of Uberon Ontology Documentation (Mungall et al. 2012, <http://obophenotype.github.io/uberone/>). Specimens were cleared and stained (C&S) following Taylor and Van Dyke (1985). An asterisk (*) indicates holotype counts. Vertebral counts included the Weberian Complex (5 vertebrae) plus all free vertebrae and the compound caudal centrum (PU1 +U1) counted as one element.

Total genomic DNA was extracted from ethanol-preserved muscle tissue of specimen CI-FML 7944 (tissue collection number: CIT-FML 00169), using a salt-based protocol (Aljanabi and Martinez 1997). A 651pb fragment of mitochondrial gene Cytochrome oxidase subunit I (COI) was amplified by Polymerase Chain Reaction (PCR) using the cocktail primers proposed by Ivanova et al. (2007). The PCR protocol was the implemented by Ward et al. (2005). PCR-amplified product was purified with 20% PEG. The product was sequenced with automated sequencer (Macrogen, Korea) in both directions to check for potential errors. The Chromatogram was processed and edited using Geneious (Technelysium Pty Ltd) and deposited in GenBank under the accession number **OQ539436**. COI sequences of different *Xylophius* species from GenBank (<http://www.ncbi.nlm.nih.gov/Genbank>) were used to assess the relationships between *X. barbatus* and the remaining species of the genus, providing a preliminary phylogenetic hypothesis. GeneBank codes and references for the employed sequences are shown in Table 2. Pairwise genetic distances with K2P model and UPGMA analysis were performed for 651 -pb fragments using MEGA11 (Tamura et al. 2021). The Maximum likelihood tree was estimated employing RAxML (Randomized Axelerated Maximum Likelihood) under GTR+FO+G4m model and 100 bootstrap replicates.

Collection's acronyms: **MACN-Ict** Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina, **MG-ZV-P** Museo Provincial de Ciencias Naturales "Dr. Ángel Gallardo", Rosario, Argentina, **MLP-Ict** Museo de la Plata, La Plata, Argentina, **CFA-IC** Fundación de Historia Natural Félix de Azara, Buenos Aires, Argentina, **CI-FML** Fundación Miguel Lillo, San Miguel de Tucumán, Argentina.

Table 1. Morphometric data of *Xyliphius barbatus*. SD= standard deviation. Holotype (MLP 6798), Paratype (MLP 2799), Paraná River (MACN-Ict 6791. 5 ex.) Bermejo River specimens (CI-FML7944 and CFA-IC-12742 34 ex.).

| | Holotype | Paratype | Bermejo River (N= 20) | | | Paraná River (N= 5) | | |
|--------------------------------------|----------|----------|-----------------------|--------------|-----|---------------------|-------------|------|
| | | | mean | range | SD | mean | range | SD |
| Standard length | 90.3 | 87.2 | 91.3 | 79.6 – 111.3 | 8.3 | 66.2 | 44.6 – 93.6 | 17.6 |
| Percentage of SL | | | | | | | | |
| Body depth at dorsal – fin origin | 15.9 | 14.1 | 14.9 | 12.3 – 16.9 | 1.2 | 17.2 | 15.6 – 19.9 | 1.7 |
| predorsal length | 42.6 | 40.4 | 40.4 | 38.3 – 45.2 | 1.4 | 41.7 | 40.0 – 43.0 | 1.1 |
| prepectoral length | 26.6 | 25.6 | 25.8 | 23.6 – 28.4 | 1.1 | 27.2 | 25.8 – 28.9 | 1.2 |
| prepelvic length | 47.3 | 45.8 | 44.0 | 41.0 – 49.1 | 1.7 | 45.9 | 43.2 – 51.8 | 3.5 |
| preanal length | 63.9 | 63.2 | 60.3 | 58.2 – 64.1 | 1.4 | 60.9 | 58.0 – 63.9 | 2.4 |
| caudal peduncle length | 26.2 | 23.2 | 27.7 | 25.0 – 31.1 | 1.8 | 26.6 | 24.4 – 29.6 | 2.0 |
| caudal peduncle depth | 7.3 | 8.1 | 7.4 | 6.8 – 8.1 | 0.3 | 8.2 | 8.0 – 8.6 | 0.2 |
| pelvic fin length | 14.9 | 12.6 | 14.1 | 12.6 – 15.2 | 0.8 | 15.5 | 13.3 – 16.6 | 1.3 |
| anal – fin base | 13.3 | 11.6 | 14.3 | 12.7 – 16.6 | 1 | 13.2 | 11.6 – 15.5 | 1.5 |
| caudal-fin length | 23.3 | 17.2 | 20.3 | 18.1 – 22.2 | 1.2 | 20.0 | 17.1 – 22.2 | 1.9 |
| pectoral spine length | 16.8 | 16.6 | 15.5 | 13.8 – 17.2 | 0.9 | 17.2 | 15.0 – 19.5 | 2.0 |
| humeral process length | 10.6 | 11.1 | 9.8 | 8.2 – 12.4 | 1.1 | 8.0 | 5.5 – 11.6 | 2.2 |
| posterior process of coracoid length | 10.4 | 9.5 | 9.8 | 8.3 – 11.1 | 0.8 | 10.5 | 9.6 – 11.7 | 0.9 |
| head length | 28 | 28.5 | 28.2 | 26.0 – 31.0 | 1.4 | 34.9 | 33.4 – 35.4 | 0.8 |
| width at pectoral – fin insertion | 27.5 | 27.7 | 24.7 | 23.3 – 27.4 | 0.9 | 27.4 | 26.8 – 28.2 | 0.6 |
| Percentage of HL | | | | | | | | |
| maximum head depth | 51.4 | 51.5 | 46.9 | 42.2 – 51.8 | 2.7 | 41.4 | 36.9 – 44.5 | 3.1 |
| snout length | 35.4 | 25.9 | 33.0 | 29.7 – 39.9 | 2.4 | 25.1 | 21.8 – 29.2 | 2.8 |
| eye diameter | 3.7 | 3.5 | 3.5 | 2.3 – 5.8 | 0.9 | 4.0 | 3.0 – 5.4 | 1.0 |
| interorbital width | 30.1 | 24.9 | 30.6 | 27.3 – 35.8 | 2.2 | 21.7 | 19.7 – 23.8 | 1.8 |
| maxillary barbel | 95.2 | 94.9 | 86.9 | 71.6 – 105.1 | 7.7 | 68.0 | 60.7 – 76.8 | 5.9 |
| anterior to posterior nares distance | 11.2 | 8.4 | 10.7 | 7.6 – 16.5 | 2.1 | 11.3 | 7.3 – 14.3 | 3.4 |
| posterior nare to orbit | 7.2 | 5.4 | 7.3 | 5.8 – 9.9 | 1.0 | 4.8 | 4.1 – 6.4 | 0.9 |
| anterior internareal distance | 20.7 | 13.8 | 16.6 | 13.8 – 22.1 | 1.9 | 15.0 | 14.0 – 15.9 | 0.7 |
| posterior internareal distance | 30.7 | 25.7 | 28.8 | 26.2 – 31 | 1.6 | 25.7 | 22.8 – 30.2 | 3.2 |

Table 2. List of sequences, with accessions and vouchers employed for molecular analysis.

| Species | GenBank ID | Catalog | Basin |
|-------------------------------|------------|----------------|---------------------------|
| <i>Xyliphius barbatus</i> | OQ539436 | CI-FML 7944 | Bermejo River. Argentina |
| <i>Xyliphius barbatus</i> | KU288948 | MG ZV-P 355 | Paraná River. Argentina |
| <i>Xyliphius lepturus</i> | MF489386 | AUM46757 | Amazon River, Peru |
| <i>Xyliphius magdalenae</i> | MF489382 | ANSP192845 | Magdalena River, Colombia |
| <i>Xyliphius melanopterus</i> | MF489383 | MUSM36715_AP12 | Amazon River, Peru |
| <i>Xyliphius melanopterus</i> | MF489384 | MUSM36715_AP28 | Amazon River, Peru |
| <i>Xyliphius melanopterus</i> | MF489385 | STRI01784 | Amazon River, Peru |
| <i>Xyliphius sofiae</i> | KU736764 | ANSP 182322 | Amazon River, Peru |

Results

Xyliphius barbatus Alonso de Arámburu & Arámburu, 1962

Figs 1–6

Material examined. All from Argentina: **MLP 6798.** Holotype. 90.3 mm SL. Paraná River at Rosario, Santa Fe province. Col. C. Vidal. **MLP 2799.** Paratype. 87.2 mm SL. Paraná River at Rosario, Santa Fe province. Col. R. Ringuelet. **MACN 6791.** 5 ex, 44.6–93.6 mm SL. Paraná River near Curtiembre, border between Santa Fe and Entre Rios provinces. 31°27'18.34"S, 60°10'11.95"W. 35–45 m depth 1961–1962 Col. N. Bellisio. **MG-ZV-P 355** (LAR-254). 1 ex, 99.1 mm SL. Paraná River in front of

Rosario, Entre Rios province, Argentina. 32°55'58.8"S, 60°37'58.8"W. 6 m depth. 04/03/2013 Col. Julián Aguilar. **CI-FML 7944.** 29 ex (3 C&S), 79.6–111.3 mm SL. **CFA-IC-12742,** 5 ex. 79,7–99,2 mm SL. San Francisco River, Bermejo River basin, Jujuy province; 23°50'27.08"S, 64°37'24.70"W, ca. 370 m asl. 1–2 m depth. 30 Sep 2016. G.E. Terán, G. Aguilera and D. Delgado.

Diagnosis. *Xyliphius barbatus* is distinguishable from the remaining species of genus by the following combination of characters: (1) seven to 11 retrorse dentations on posterior margin of pectoral-fin spine (vs. six in *X. anachoretetes* and four or five in *X. magdalenae*); (2) 24 to 30 dendriform papillae on inferior lip (vs. 20–22 in *X. magdalenae*, 30 in *X. sofiae*, and 22 to 27 triangular papillae, with only the lateral ones branched in

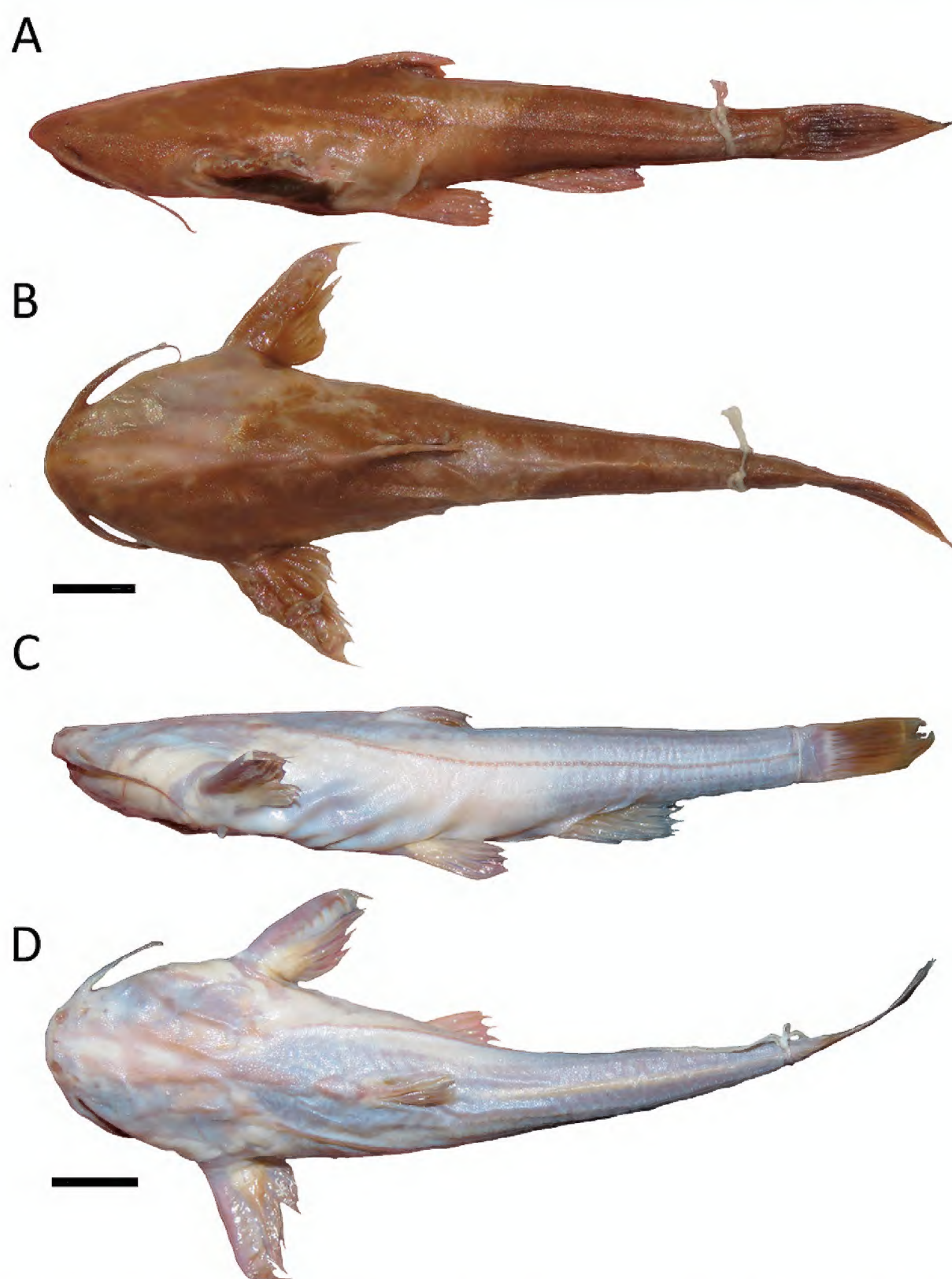


Figure 1. A, B. Holotype MLP 6798 of *Xyliphius barbatus*. 90.3 mm SL. C-D Paratype MLP 2799. 87.2 mm SL. Paraná River at Rosario, Santa Fe province, Argentina. Scale bar: 10 mm.

X. kryptos); (3) I,3 or I,4 dorsal-fin rays (vs. I,5 in *X. lepturus* and *X. melanopterus*); (4) absence of dorsal pale band from snout tip to caudal-fin origin (vs. presence in *X. anachoretas*, *X. magdalenae* and *X. melanopterus*); (5) absence of a latero-dorsal band following the second row of tubercles on anterior part of body (vs. present in *X. magdalenae* and *X. melanopterus*); (6) eyes present and reduced (vs. absent in *X. sofiae*); (7) five to eight anal-fin rays (vs. nine in *X. lepturus*).

The additional characters that distinguish *Xyliphius barbatus* from the remaining species of the genus are: papillae dendriform on lower lip with large branches (vs. papillae with minute branches on *X. anachoretas*); three dorsal procurrent rays (vs. two in *X. anachoretas*, four to five in *X. lepturus*, and four in *X. magdalenae*); pelvic fin not reaching anal-fin origin (vs. just reaching in *X. magdalenae*); maxillary barbel surpassing pectoral-fin spine insertion (vs. not quite reaching pectoral in *X. magdalenae*); four branchiostegal rays (vs. five in *X. sofiae*); and two ossified proximal radials on pectoral fin (vs. one in *X. sofiae*).

Morphological description. Morphometric data is summarized in Table 1. Head and anterior part of body depressed, compressed from dorsal-fin origin to caudal-fin insertion. Maximum depth at dorsal-fin origin. Dorsal profile straight from snout tip to dorsal-fin origin, relatively depressed along dorsal-fin base, and relatively straight from this point to caudal-fin origin. Ventral profile of body, straight from lower-jaw to pectoral-fin origin, convex to pelvic-fin origin with the lowest point at the origin of the pelvic bone, then slanted dorsally to anal-fin origin and relatively straight from this point to caudal-fin base. Greatest width just anterior to pectoral-fin origin.

Head triangular with a rounded snout. Eyes reduced (Fig. 3), located closer to snout tip than to supraoccipital protuberance, and covered with skin less pigmented than surroundings areas. Two nares, anterior nostril tubular, posterior one smaller and located closer to eyes than to anterior nostril. Maxillary barbel on side of snout, inserted just above rictus. Maxillary barbel reaching pectoral-fin base. Two pairs of mental barbels smaller than maxillary ones and located close to the mouth gape.



Figure 2. Lateral, dorsal and ventral views of *Xyliphius barbatus*. CI-FML 7944. San Francisco River, Bermejo River basin, Jujuy province. Scale bar: 10 mm.

The external one just before the vertical line through eye, reaching mouth gape when adpressed; inner mental barbels smaller, reaching outer barbels origin, surpassing the lower lip and reaching end of papillae when adpressed. Mouth subterminal, much wider than snout, with 20 to 30 dendritic papillae. Anterior margin of snout with a groove at middle line in dorsal view. Gill slits small, located ventrally on head, before pectoral-fin origin; gill membranes united to isthmus. Small genital papillae just posterior to anus.

Head, trunk, and fins are all covered by thick skin, while the skin in the ventral area and at the fin insertions is thinner. Trunk covered by unculiferous tubercles, which are more concentrated in the head. Five lateral rows of large tubercles extending from post-cephalic region to caudal-fin base, and concentrated in mid-dorsal line.

Dorsal-fin I,2,i (1) or I,3,i (33*), spine feeble. Dorsal-fin insertion on anterior half of body, closer to snout tip than to caudal-fin insertion, anterior to pelvic-fin insertion; shape triangular, rays elongated beyond the membrane. Anal fin i,3,i(1), ii,3,i(6), iii-3,i(7), ii-4,i(16*),

iii-4,i(1), ii-5,i(3), ovoid, first branched anal-fin ray longest. Anal-fin insertion on the posterior half of body, closer to caudal-fin insertion than to snout tip. Pectoral-fin I,4,i (34*), its origin almost at half way between snout tip and dorsal-fin origin, and its distal tip reaching pelvic-fin origin. Distal tips of branched rays elongated beyond membrane. Pectoral spines thick, with seven to 11 developed retrorse serrae along posterior margin; spine capped by a fleshy elongation. Pelvic fin i,3,i (1) or i,4,i (33*), origin just posterior to vertical through dorsal-fin origin. The tip of pelvic-fin rays elongated throughout the membrane. Caudal fin i,4/4,i (34*), The ventral-most three branched caudal-fin rays longer.

Osteology (Figs 4–6).

Mesethmoid deep, slightly longer (anteroposteriorly) than wide, with an anterior notch separating two anterior wings. Premaxillae articulating on a ventrolateral concavity of mesethmoid, not visible in dorsal view due to a dorsal lamina. Two diverging laminae articulating dorsally with frontals by interdigitations. Lateral ethmoids articulate with frontals through a dorsal interdigitated



Figure 3. Head detail of *Xylophius barbatus*. CI-FML 7944. San Francisco River, Bermejo River basin, Jujuy province. Scale bar: 5 mm.

process and medially with orbitosphenoid by cartilage; the latter covered ventrally by a laminar extension of the same bone articulating with parasphenoid; projecting lateral process joined to autopalatine at its middle length; ventrally flattened and extensively articulating with parasphenoid posteromedially. Frontals about 3.5 times longer than wide, their posterior wing articulating with supraoccipital, and lateral posterior margins enclosed by anterior extension of sphenotic. Anterior fontanel about 1.4 times larger than posterior one, synchondral articulation between lateral ethmoids and mesethmoid completely visible through fontanel. Supraoccipital enclosing almost half of posterior fontanel. Epiphyseal bar with a strong suture, its length equal to or greater than that of posterior fontanel. Supraoccipital a little longer than wide, extensively articulating with pterotic laterally, and with epioccipital posterolaterally; a notch in posterior region receiving the ascending process of posttemporal-supracleithrum; posterior process wide and short, in contact with dorsal portion of complex vertebrae. Sphenotic pitted; ventral surface with greater pores, sutured to pterotic laterally, leaving a posterior space with same bone in ventral view, with extensive anterior synchondral articulation with hyomandibula, sutured medially to parasphenoid and posteriorly to anterior lamina of prootic. Dorsal prootic covering lateral surface of frontals and with posterior articulation with supraoccipital. Pterotic with anterior lamina sutured to sphenotic, a concavity after its contact with suprapreopercle, and a lateral rounded expansion reaching opercle ventrally; contacting posttemporal-supracleithrum lateral arm at its posteriormost region. Epioccipital articulating anterolaterally with pterotic, medially with supraoccipital and dorsally with posttemporal-supracleithrum. The latter bone with dorsal process reaching lateral surface of supraoccipital over epioccipital; a ventral pointed process contacting posterior expansion of pterotic and posterior arm of the bone sutured to complex vertebra. Wider portion of parasphenoid at its articulation with sphenotic, extended to middle basioccipital at its posterior end. Prootic visible only in ventral view, anterior lamina over sphenotic cartilage, extensively contacting parasphenoid medially,

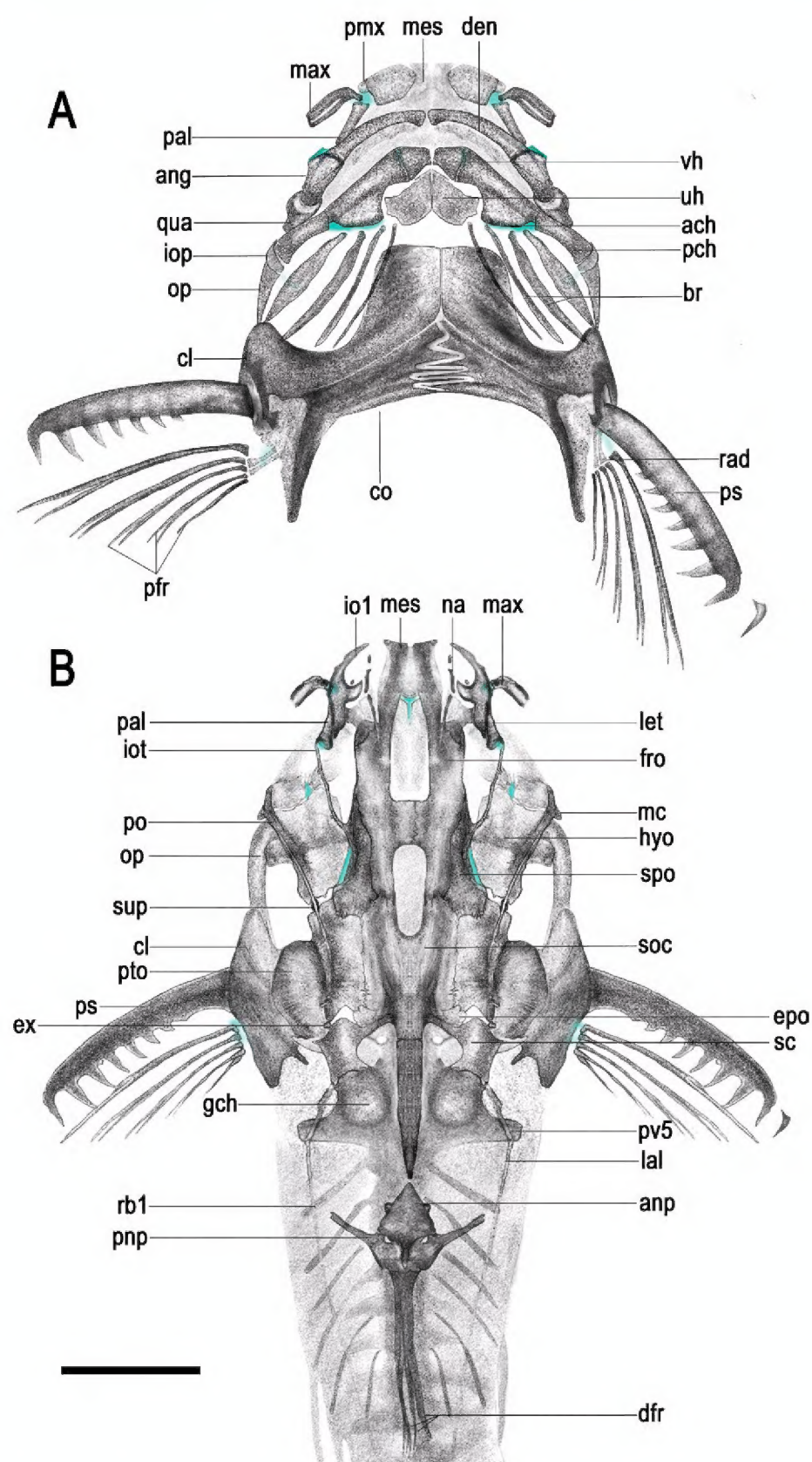


Figure 4. Skull of *Xylophius barbatus* CI-FML 7944. **A.** Ventral view; **B.** Dorsal view. ach: anterior ceratohyal; ang: anguloarticular; anp: anterior nuchal plate; br: branchiostegal rays; cl: cleithrum; co: scapulocoracoid; den: dentary; dfr: dorsal fin rays; epo: epioccipital; ex: extrascapular; fro: frontal; gch: gas bladder chamber; hyo: hyomandibula; io1: infraorbital 1; iop: interopercle; iot: infraorbital tubules; lal: lateral line tubules; let: lateral ethmoid; mc: mandibular canal tubules; mes: mesethmoid; max: maxilla; na: nasal; op: opercle; pal: autopalatine; pch: posterior ceratohyal; pfr: pectoral-fin rays; pmx: premaxilla; pnp: posterior nuchal plate; po: preopercle; ps: pectoral-fin spine; pto: pterotic; pv5: parapophysis of vertebra five; qua: quadrate; rad: pectoral-fin radial; rb1: first rib; soc: supraoccipital; sc: posttemporal-supracleithrum; spo: sphenotic; sup: suprapreopercle; uh: urohyal; vh: ventral hypohyal. Scale bar: 1 mm.

and sutured to pterotic posterolaterally, leaving to an anterior space with the same bone. Exoccipital bearing two projections enclosing posterior parasphenoid, strongly sutured to posteromedial edge of prootic, to exoccipital laterally and with complex vertebra by interdigitations. Basioccipital sub triangular, with a large foramen on its posterior half, a conspicuous pore anteromedially and additional ones laterally.

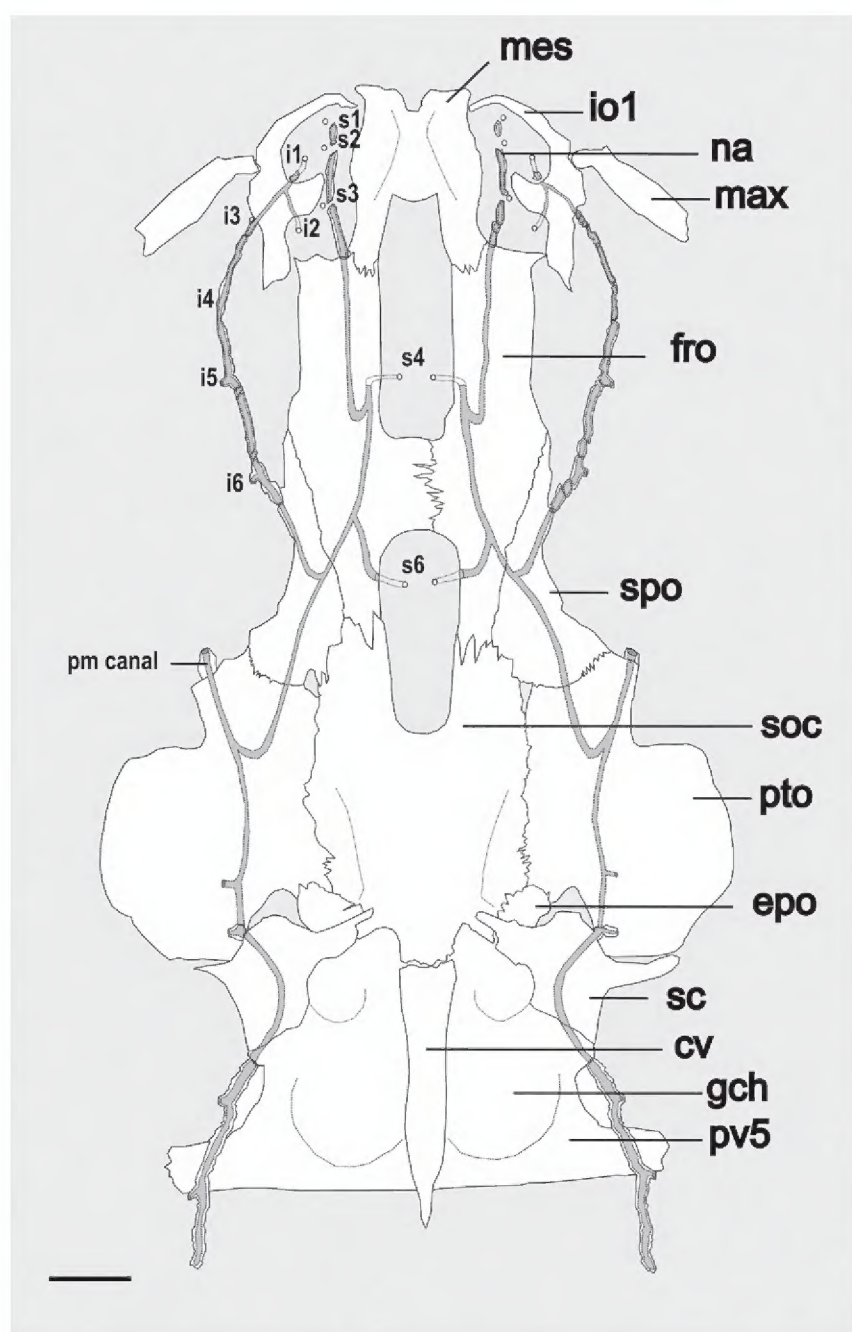


Figure 5. Dorsal view of the neurocranium of *Xyliphius barbatus* CI-FML 7944. mes: mesethmoid; io1: infraorbital 1; na: nasal; max: maxilla; fro: frontal; spo: sphenotic; soc: supraoccipital; pto: pterotic; epo: epioccipital; sc: supracleithrum; cv: weber camara; gch: gas bladder chamber; pv5: parapophysis of vertebra five. Pores of the cephalic sensory lateral system s1 to s6 represent the pores from the supraorbital canal and i1 to i6 from the infraorbital canal. Scale bar: 2 mm.

Premaxillaries dorsomedially flattened and oval, in contact with ventrolateral notches of mesethmoid and separated by the latter. Premaxillary teeth absent. Maxillary tubular, with furrowlike opening on ventral surface; its condyle attached to anterior palatine cartilage. Dentary long and slender, laminar posterior region overlapping anterior face of angular; teeth conical and pointed inwards, arranged in two rows, the outer one with 3 to 5 teeth near the symphysis, and the inner row with 11–12 teeth; coronomeckelian absent. Meckel's cartilage somewhat conical, wider laterally from its origin at angular, and slender medially at its joining with the dentary, which is located ventral to (or a little displaced medially) dentary dorsolateral notch.

Hyomandibula in dorsoventrally oblique position with respect to neurocranium; dorsalmost edge under anterior extension of sphenotic, followed by a synchondral articulation with the same bone; anterior cartilage contacting quadrate and extended to lateral portion of metapterygoid. Preopercle on lateral portion of hyomandibula, sutured to quadrate on its synchondral articulation with the same bone. Quadrate condyle anteroventrally oriented, to anguloarticular. Metapterygoid square, a little larger than endopterygoid, with a posterior concavity for the anterior lamina of hyomandibula. Endopterygoid ventral to posterior third of autopalatines, at posteroventral concavity of lateral ethmoids after its projecting lateral process, bearing a lateral pointed projection reaching autopalatine middle cartilage.

Autopalatines with expanded anterior and posterior edges, its narrower portion anterior to lateral ethmoids cartilage. Opercle medially articulated with lateral arm of hyomandibula, anteriorly expanded and sutured with interopercle; posterior pointed projection reaching ventral expansion of pterotic. Interopercle accompanying ventralmost edge of opercle, its anterior pointed projection over posterior ceratohyal, covering the interhyal articulation from lateral view.

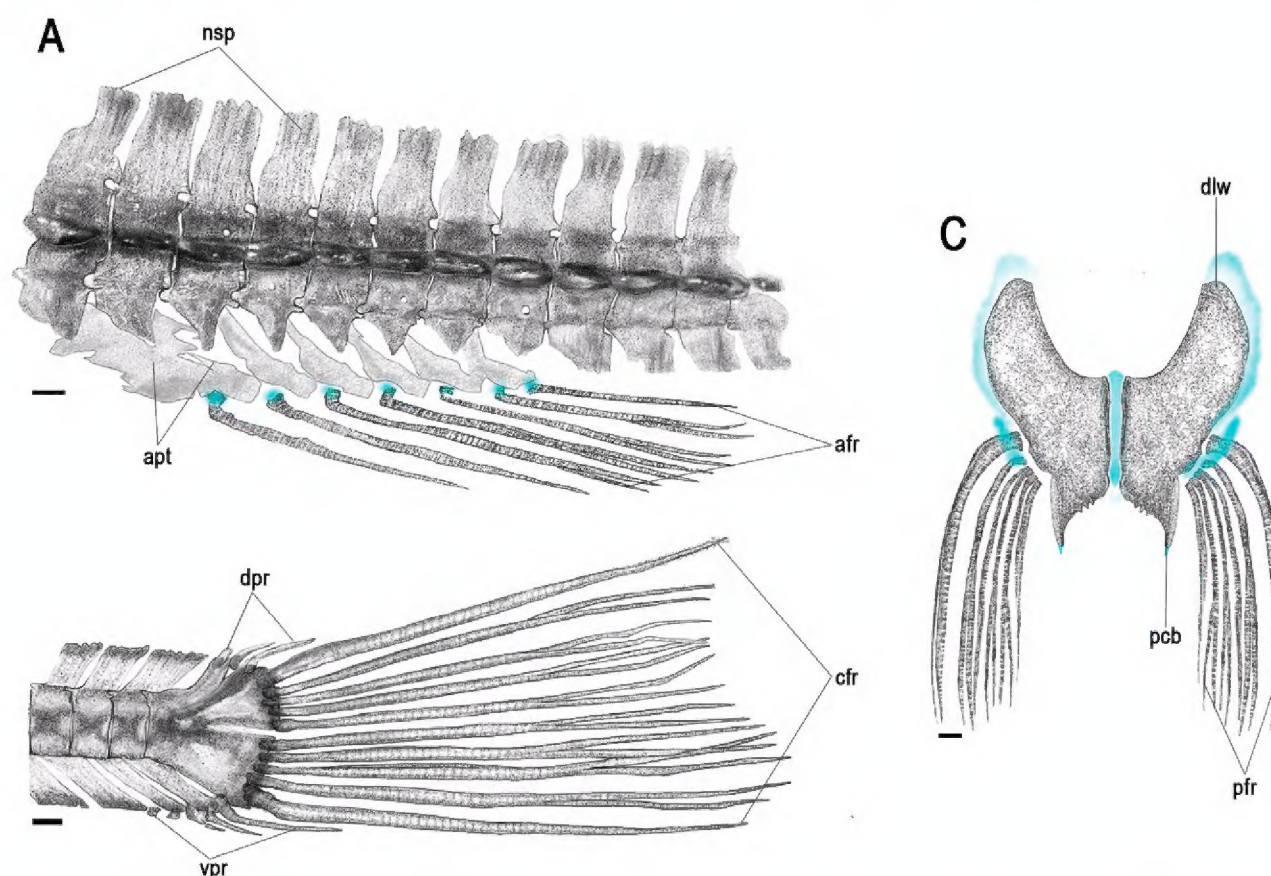


Figure 6. Anal, caudal and pelvic fins of *Xyliphius barbatus* CI-FML 7944. **A.** Anal-fin and pterygiophores; **B.** Caudal complex and fin; **C.** basipterygium. afr: anal-fin rays; apt: anal-fin pterygiophores; cfr: caudal-fin rays; dlw: dorsolateral wing of basipterygium; dpr: dorsal procurent rays; nsp: neural spines of vertebrae 15 to 18; pcb: posterior cartilage of basipterygium; pfr: pelvic-fin rays; vpr: ventral procurent rays. Scale bar: 1 mm.

Urohyal subtriangular, with a medial longitudinal cleft on anterior corner and posterolateral developed wings. Two basibranchials, the anterior one about twice larger, contacting first hypobranchial anteriorly and second hypobranchial cartilage posterolaterally; posterior one reached by third hypobranchial. Only the first hypobranchial ossified with wider distal portion. Hypohyals squarish, narrow in its proximal region, and articulated to ceratohyal synchondrally. Ceratohyals with posteroventrally expanded lamina at the articulation with branchiostegal rays; dorsal and ventral extensions over cartilage with posterior ceratohyal sutured to anterior extensions of same bone. Posterior ceratohyal rectangular, with a notch anterior to posterodorsal corner at the articulation with interhyal. Branchiostegal rays four, lateral ones thicker and with more developed lamina, first one (medial) between posterior urohyal and lateral to corner of ceratohyal expanded lamina, the remaining ones associated with anterior ceratohyal cartilage, lateral-most at the articulation cartilage of ceratohyals. Interhyal present, articulated to posterodorsal ceratohyal, with lateral portion of hyomandibular and quadrate.

Ceratobranchials five, first two and last one (fifth) with a single series of small gill rakers, third and fourth with two series; fifth ceratobranchial bearing a dorsal drop-shaped plate with conical teeth, posterior portion long and slender, with four or five gill rakers. Five gill rakers on the anterior border of first and second ceratobranchials, one in the cartilage with first epibranchial; only epibranchials one to three with gill rakers, first with single row and the remaining two with double row of one or two gill rakers restricted to proximal portion. Epibranchials four, an uncinate process on the third one. Third pharyngobranchial thicker at its articulation with cartilage of third epibranchial; fourth pharyngobranchial about half of the latter and located dorsal to an oval tooth plate.

Nasal separated in two tubular ossifications by supraorbital sensory pore s2, posterior tubular ossifications of the supraorbital canal enters frontal just lateral to its articulation with mesethmoid posterior projection. Antorbital present, small, its canal piercing base of dorsal projection of infraorbital 1 and exiting posteriorly. First infraorbital over anterior cartilage of autopalatine, with notch bordering maxillary condyle; anteromedial projection pointed and curved, limiting the anterolateral portion of nares. Posterior infraorbitals as a small series of ossicles entering sphenotic canal laterally; ventral branches of i5 and i6 ossified.

Supraorbital sensory canal with pores s1-s2 and s2-s3 separating nasal in two tubular ossifications. Pore S4 opening at anterior frontal fontanel, s5 is missing and s6 opening at posterior frontal fontanel. Infraorbital sensory canal composed of six pores, the first and second ones opening at inner margin of infraorbital 1, and the third at outer margin. Pores i4 to i6 arranged in an arch reaching up close to anterior half of sphenotic.

Tubular series of preopercle mandibular canal initiating below posterior portion of dentary and separated by

those joined to preopercle by a gap just lateral to quadrate condyle. Suprapreopercle as a small tubular canal between hyomandibula and pterotic, with dorsal and ventral laminae present. Extrascapular present. Lateral line complete, beginning at posterolateral exit of posttemporal-supracleithrum canal.

Dorsal lamina of Weber apparatus reaching dorsal surface, with flattened and slender process almost reaching nuchal plate posteriorly. The latter rhomboid in dorsal view, with a ventral lamina reaching neural processes of sixth vertebra. Posterior nuchal plate with two slender and pointed anterolateral projections joined to first rib by a ligament, ventrally contacting anterior plate. Gas bladder chamber evident from dorsal view, posteroventral portion partially enclosed by an anteriorly directed ventral lamina. Parapophyses of fifth vertebra reaching lateral wall of body, continuous with posterolateral edge of cleithrum; extensively joined to posterior region of complex vertebrae and covering its lateral border. Ribs six, first pair on sixth vertebra. Vertebrae 35, first 30 or 31 bearing transversal lateral processes.

Pectoral spine retrorse serrations larger distally; first branched ray reaching end of spine or slightly beyond. Two proximal radials associated with the three proximal most rays; first branched ray associated with scapulocoracoid cartilage. Cleithrum dorsomedial pointed projection entering cavity formed by ventral posttemporal-supracleithrum and lateral lamina of complex vertebra; dorsolateral arm with a proximal pointed projection lateral to posttemporal-supracleithrum and rounded edge; medial arm anteriorly concave, bearing an extensive contact scapulocoracoid posteriorly, and sutured to contralateral cleithrum in larger C&S specimen (102.58 mm SL). Coracoids strongly interdigitated medially, their posterior processes passing base of last pectoral-fin rays and reaching vertical through dorsolateral arm of cleithrum. Pelvic fins not reaching anal-fin origin. Basipterygia with developed dorsolateral wings and lateral cartilage present; medial cartilage not reaching posterior medial margin of bone, which bear jagged borders; posterior cartilage short. First anal-fin pterygiophore reaching posterior portion of vertebra 15 and posterior anal-fin pterygiophore at posterior portion of vertebra 21. Caudal fin with five principal rays on both lobes, three dorsal and four or five ventral procurent rays.

Coloration (Fig. 7).

Ground of body dark brown to black, head light brown, pectoral region lighter than dorsal region. A barely evident light brown middorsal stripe on head from snout tip to the middle of caudal peduncle, interrupted at dorsal-fin base. Lateral rows of tubercles brown, lighter than the background. Maxillary barbels dark brown with lighter tips; the remaining barbels light brown. Pectoral fins black with whitish tips; anal fin black with distal half whitish; all the other fins black with the distal third whitish. After the fixation process, the color tends to become paler brown, and the white portions on the fins are less noticeable (Figs 1–3).



Figure 7. CI-FML 7944. *Xyliphius barbatus*. Live specimen, 92.4 mm SL. San Francisco River, Bermejo River basin, Jujuy. Scale bar: 10 mm.

Molecular analysis. Molecular comparison employing the COI sequence (see Table 2) shows no difference between *Xyliphius barbatus* specimen from lot CI-FML 7944 and *Xyliphius* sp. reported by Díaz et al. (2016) from Paraná River Basin in Rosario, Argentina, herein identified as *Xyliphius barbatus*. The estimated evolutionary divergence (number of base substitutions per site from between sequences) is quite small ($D \geq 0.0031$) between *X. barbatus* and *X. melanopterus*, but greater with specimens of *X. magdalenae*, *X. sofiae* and *X. lepturus* ($D=0.1092$; 0.1414 and 0.1558). Comparisons with *X. anachoretas* and *X. kryptos* were not possible due the lack of available COI sequences for these species. Moreover, the tree topology for UPGMA and Maximum Likelihood analysis was similar (Fig. 8).

Distribution. Including the new record from the San Francisco River, upper Bermejo River basin, Jujuy province and the previous records from the Paraná River, in Rosario (Santa Fe province), and in Chaco province (locality of *X. lombarderoi*), together with the records from the Paraguay River basin in Paraguay reported by Carvalho et al. (2017) and from the Pantanal in Brazil (Gimênes Junior and Rech 2022), the species exhibit a broad distribution in the Parana-Paraguay system. The new record of specimens from the San Francisco River, Upper Bermejo River basin, Jujuy province, is approximately 750 km in a straight line from the closest record in Tragadero River, Paraná River basin in Chaco Province (Fig. 9).

Ecological notes. Most of the records of this species are from the main channel of big rivers and were collected by trawl nets from the bottom of Parana River at 35–40 m depth (MACN 6791). The specimens from the San Francisco River (Fig. 10) were collected (1 to 4 m depth) using cast nets, and hand nets. Other informal captures made by anglers, supported by photographic evidence (see Suppl. material 1), include one record from the Paraná River in Misiones province (6 to 8 m depth), and another record in Misiones, in the Bermejo River, Salta province (about 4 m depth). In both cases, captures were made using earthworm as bait (Julio Endler and Roberto Toval), see appendix 1. Although speculative, based on available records, this species seems to exhibit fossorial habits, regardless of the substrate depth.

Conservation status. The global conservation status of *Xyliphius barbatus* was evaluated in 2021, being considered as Near Threatened under criteria B2a by the IUCN

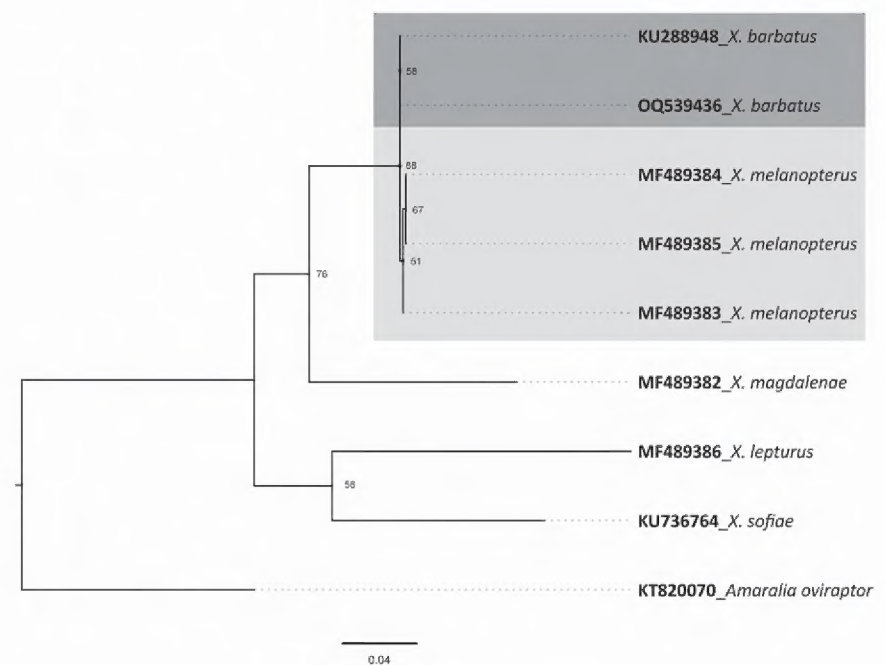


Figure 8. Maximum likelihood tree based on available COI sequences. Bootstrap values are shown next to each node. The boxes highlight the close relation between *X. barbatus* and *X. melanopterus*.

Red List of Threatened Species (Vera-Alcaraz 2023). When the species was evaluated, there was registers for only three specific locations at the Paraná and Paraguay rivers, with an estimate of the Extent Of Occurrence (EOO) of 21,366 to 143,190 km², and an estimate of Area Of Occupancy (AOO) between 48 km² and 1,999 km². According to these values, the species belongs to threat categories (criterion B2), but without meeting sufficient conditions to be considered as a species threatened with extinction. Since its evaluation, four new localities have been found, three in Mato Grosso de Sul, almost 600 km farther north from the nearest locality (Gimênes Junior and Rech 2022), and the locality reported in the present work, more than 650 km to the west of the nearest locality, which significantly expands the known distribution of the species. A preliminary exploration of the data indicates an EOO of about 600,000 km², which greatly exceeds previous estimates. A detailed reassessment of the species' threat status under IUCN criteria is necessary, which will likely result in a recategorization to Least Concern (LC).

However, evaluations for this species should be made with caution because it has relatively few records and inhabits areas that are difficult to sample. Additionally, there is no information available on its population structure or density throughout its range.

Comparative material examined. See Suppl. material 2.

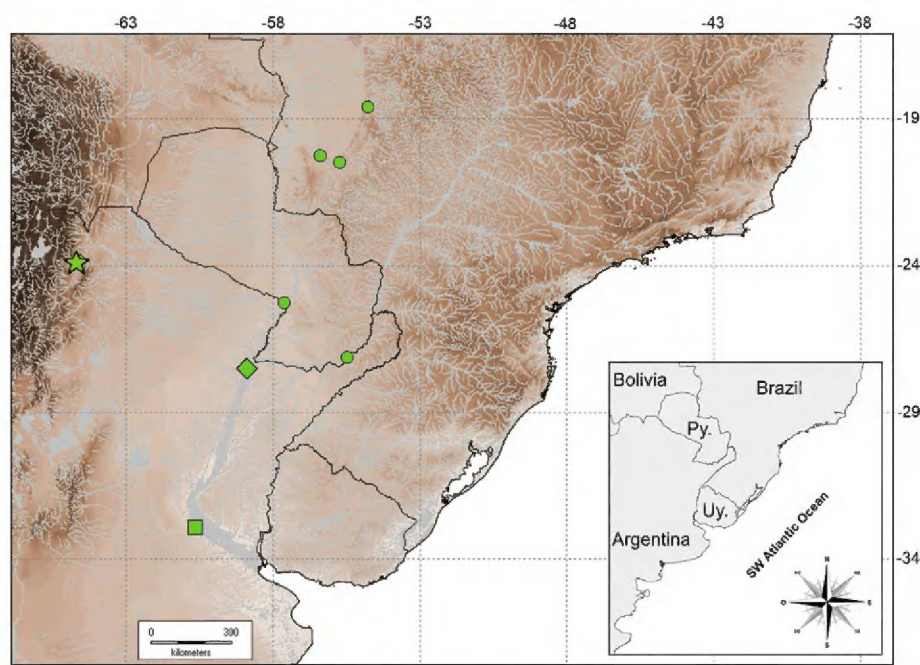


Figure 9. Geographical distribution of *Xylophius barbatus*. Square: type locality of *Xylophius barbatus*; rhombus: type locality of *Xylophius lombarderoi*; Circles: other published records; star: new record from Bermejo River.

Discussion

In this contribution we report the northwesternmost record of *Xylophius barbatus* in Argentina, more than 750 km from the previously distribution record of the species. According to Carvalho et al. (2017) species of *Xylophius* are commonly found in the main channel of large rivers and most records are restricted to the upland portions of Andean piedmont rivers. Conversely *X. barbatus* and *X. sofiae* occur in relatively lowland stretches. Records of these species and their presence in museum collections are scarce, mainly due to the depth at which they occur, making them difficult to sample. Additionally, their fossorial habits and mimetic coloration contribute to this scarcity. *Xylophius barbatus* was also recorded in the Uruguay River basin by Loureiro et al. (2013), but this record is indeed a miss-identification of *Bunocephalus doriae* (Loureiro Pers. Comm., 2022). García (1992) includes *X. barbatus* in a list of species from the Paraná River basin in Misiones, but the accuracy of this record cannot be confirmed because the reference specimen is missing; in addition, this record is part of the environmental impact report and is not a formal publication. Nevertheless, the information provided by García (1992) was subsequently repeated (Liotta 2006; Calviño and Castello 2008; Rosso and Liotta 2021).

The scarcity of material has led to biased descriptions, since variations in morphology and meristic counts have not been considered, making it difficult to establish comparisons among species. This situation was also highlighted by Figuereido and Britto (2010) in the description of *Xylophius anachoretas*; the authors considered that any inferences concerning the direction of change in the number of papillae from lower-lip is speculative and premature due to the absence of comparative material.

The specimens of *Xylophius barbatus* collected in the upper Bermejo River represent the largest known batch for the species and one of the largest for the genus. Other numerous collections of aspredinids were made under



Figure 10. San Francisco River, Jujuy province at 23°50'27.08"S, 64°37'24.70"W.

special conditions. For example, when a river section was dried 70 exemplars of *Hoplosternon sexpapilostoma* Taphorn and Marrero 1990 were collected, and when a dam was closed more than 100 exemplars of *Xylophius* cf. *lepturus* were collected (Taphorn and Marrero 1990). This suggests the low efficacy of conventional fishing methods due to the distinct habitat use by members of the genus (Carvalho et al. 2017). The new *Xylophius barbatus* material obtained has allowed us to make an accurate re-description for a species which, despite its wide distribution in the Paraná-Paraguay River basins, is poorly represented in fish collections in museums. The inclusion of new material for the re-description of *Xylophius barbatus* has led to an objective assessment of inter-specific variation of characters useful for distinguishing the species from the remaining members of the genus. Among these characters, we found that the lower limit of papillae on lower lip ranges from 24 to 30 instead of the previously reported 28 to 30; the anal-fin rays now range from 5 to 8 instead of 6 to 8; the number of retrorse serrae along the posterior border of the pectoral-fin spine now ranges from 6 to 11 instead of 6 to 8; and the extension of lower limit range of vertebral count to 35.

From a genetic perspective, maximum likelihood analysis identified two main groups: one comprising *Xylophius lepturus* and *X. sofiae*, and the other including *X. magdalenae*, *X. barbatus*, and *X. melanopterus*. This tree topology aligns with the morphological inferences made by Carvalho et al. (2017). They suggested that *X. sofiae* and *X. lepturus* shared enough features to support a sister-group relationship. These characters include snout morphology, lack of median notch, fifth ceratobranchial morphology, branchial apertures separation, anal-fin rays' modal number; and lateral line extension. Carvalho et al. (2017) also proposed a close relationship between *X. anachoretas*, *X. barbatus*, and *X. melanopterus* who share a relatively long and narrow fourth parapophysis and fifth parapophysis deflected anteriorly (vs. short and broad fourth parapophysis and fifth directed laterally in the remaining species of *Xylophius*). Despite the lack of

X. anachoretas sequences, our ML tree recovers a very close relationship between *X. barbatus* and *X. melanopterus*. The low genetic distance between *Xyliphius barbatus* and *X. melanopterus* is noteworthy, since the distance to other species and between the other species is almost 50 times that value. In fact, one of the sequences of *X. melanopterus* (MF489383) differs only in 1 nucleotide from the *X. barbatus* sequence. It is evident that this comparison (based in one mitochondrial marker and a few sequences) is not enough to make inferences about the taxonomy of these two species (which have morphological differences, such as ray counts and coloration, and inhabit different basins), but it highlights the need for a complete morphological and molecular revision of the group in order to complement its definition and species diagnosis.

Additional similarities in the complex vertebra between *Xyliphius melanopterus* and *X. barbatus* were previously indicated by Carvalho et al. (2017); a narrow and long fourth parapophyses vertebra (observed in examined specimens), and anteriorly deflected fifth parapophyses, perpendicular in the examined *X. barbatus*. The same authors discuss the possible independent acquisition of gas bladder encapsulation in *Xyliphius* due to its absence in *X. magdalenae* and *X. kryptos* (see also Carvalho et al. 2018, Fig. 3), and the presence in the remaining congeners (with the polymorphism in *X. lepturus*). Our results also suggest the reduction of this feature in *X. magdalenae* instead of two independent events of gas bladder encapsulation (Fig. 6A). Nonetheless, these characters must be tested and optimized in a morphological analysis to support any conclusive hypothesis.

Neither Friel (1994) nor Cardoso (2008) included specimens of *Xyliphius barbatus* in their works. *Xyliphius barbatus* bears the eight synapomorphies proposed by the former author and the additional six synapomorphies for the subfamily Xyliphiinae proposed by the latter author: (1) lateral surface of frontal lacking orbital concavity; (2) antero-dorsal process of lacrimal-antorbital (first infraorbital) developed; (3) supra-preopercle present Friel (1994: Ch.28); (4) expanded proximal margin of posterior ceratohyal (Friel 1994: Ch.34); (5) more than 30 gill filaments on first epibranchial and ceratobranchial (Britto 2002: Ch. 126); (6) four to eight dorsal plus ventral procurent rays on caudal-fin. We also observed the bony bulge on ventral surface lateral ethmoid possibly housing the olfactory bulb as suggested by Carvalho et al. (2017), as an additional synapomorphy of the genus.

Conclusions

In this work, an accurate re-description of *Xyliphius barbatus* based on osteological observations, morphometry, meristic counts and molecular data is provided. The distributional range of this species is widened to the upper Bermejo River basin in northwestern Argentina, more than 750 km in straight line from the previously known

record of the species in the country. A provisional phylogenetic molecular hypothesis is provided in which the close relation with *X. melanopterus* is observed.

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Supplementary material 1

Informal records by anglers, with photograph evidence. Specimens collected with earthworm bait

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Data type: docx

Explanation note: The information provided here represent records of *Xyliphius barbatus* capture by anglers in two localities along the Paraná river, also including a map of these localities.

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Supplementary material 2

Comparative material examined

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Data type: docx

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